Amino Acid and Protein Incorporation in Water-Stressed Perennial Ryegrass

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Amino acid and protein incorporation in water-stressed perennial ryegrass

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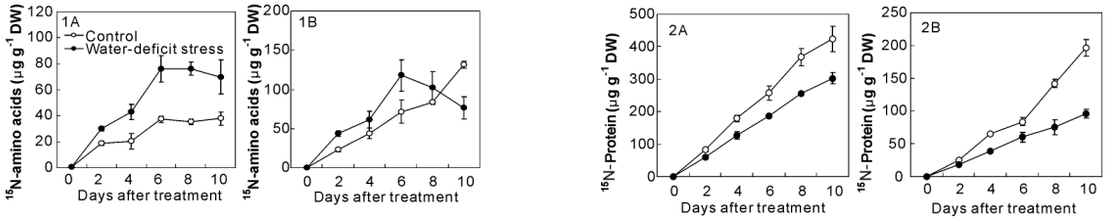
Introduction During prolonged periods of drought , the decrease in water availability for transport-associated processes leads to changes in the concentrations of many metabolites , followed by disturbances in amino acid and carbohydrate metabolism . In particular , changes in the concentration and composition of the N-soluble fraction in response to both water-deficit (Girousse *et al* . 1996 ; Lazcano-Ferrat and Lovatt 1999) and salinity stress (Gilbert *et al* . 1998 ; Silveira *et al* . 2001) have been demonstrated in a wide range of species . Although there have been many studies of the physiological and molecular processes that enable the plant to tolerate drought stress and of nutrient acquisition during water deficit , little information is available concerning the kinetics of *de novo* amino acid and protein synthesis in relation to increasing stress intensity over time , during water deficit . The present work was designed to investigate the kinetic patterns of *de novo* protein and amino acid synthesis in response to increasing intensity of water deficit stress .

Materials and methods $^{15}NO_3^-$ feeding was carried out daily throughout a 10 day experimental period . For ^{15}N feeding for the well-watered (control) treatment , 25 mL of ^{15}N solution (1 mM K$^{15}NO_3$ with 8 .34 ^{15}Natom % excess) was administered evenly through three porous plastic tubes buried vertically to a depth of 5 cm in each pot at 10 :00 h and 16 :00 h , respectively . Using the same protocol of administration , plants submitted to water-deficit received 2 .5 mL of ^{15}N solution , containing the same ^{15}N atom % and the same amount of N as applied to the control pot (i .e 0 .7 mg N pot^{-1} d^{-1}) . Amino acid and protein were fractionated as described by Kim *et al* . (2004) . N content and ^{15}N atom % of all fractions were determined using N single mode analysis on an ANCA-SL isotopic ratio mass spectrometer (PDZ-Europa , Crewe , UK) . Samples were harvested at 0 , 2 , 4 , 6 , 8 , and 10 days after treatment .

Results In the leaves under water deficit conditions , the amount of newly absorbed N in the amino acid fraction (^{15}N-amino acid) increased much more rapidly during the initial 6 days and was 2 .0-fold higher at day 6 compared to the control . The levels were continuously maintained until day 10 (Figure 1A) . In roots , water deficit increased the ^{15}N-amino acid for the initial 6 days (+65% compared with the control at day 6) , and then declined to 58 .2% of control values at day 10 (Figure 1B) . The amount of N derived from the newly absorbed N in the protein fraction (^{15}N-protein) in both leaves and roots increased linearly , but the absolute level was significantly lower in water deficit plants . The amount of ^{15}N-protein in water deficit stressed leaves was 27 .2% lower than control after 6 days of water deficit treatment . The ^{15}N-protein level continued to decrease relative to control until day 10 (71 .3% , Figure 2A) . *De novo* protein synthesis in roots was significantly lower in water deficit (-51%) than in control plants at day 10 (Figure 2B) . These results show that drought stress significantly increased ^{15}N-amino acid synthesis while it decreased ^{15}N-protein synthesis . These results suggest that the accumulation of amino acid is possibly due to *de novo* synthesis rather than protein degradation .



Conclusions The present data clearly indicate that the increase in *de novo* amino acid synthesis caused by water deficit is a transient adaptive response for the early period , which is mainly due to the decrease in *de novo* protein synthesis .

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